











Worklist: 3931

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2019-2347	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2351	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2361	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2365	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2394	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2401	6	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2019-2432	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2019-3807	1	BCK	AM 27 Blood THC Quant by LC-QQQ	



AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 1/7/20
Plate lot#: 190716

Analyst: Anne Nord
Plate Expiration: 1-16-2020

Mobile phase A: 0.1% Formic Acid in LCMS Water MTBE
Mobile phase B: 0.1% Formic acid in Acetonitrile Hexane
LCMS Methanol

Blank Blood Lot: 19H52275 **Urine Blank:** 11719 **Column:** UCT Selectra DA 100 x 2.1mm 3um
LCMS-QQQ ID: 69679

Pre-Analytic:

- 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.


Analytic:

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Urine hydrolysis: add 1.5 ml urine to blank plate, add 250 ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
Pipette 1000µL blood (calibrated pipette) Pipette ID: k52558g in wells of analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
- 4. Pipette 500µL 0.1% formic acid in water blood sample, 500 ul saturated phosphate buffer in urine in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer 800µL of blood+acid or urine acid mixture to corresponding wells of SLE+ plate.
- 7. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent).
(Load at 85-100 PSI- Selector to the right) Manifold ID: 66792
- 8. Wait 5 minutes.
- 9. Add 2.25mL MTBE. *(Add in 3 increments of 750uL)*
- 10. Wait 5 minutes.
- 11. Apply positive pressure for approx. 15 seconds. *(10-15 PSI- Selector to the left).*
- 12. Add 2.25mL Hexane. *(Add in 3 increments of 750uL)*
- 13. Wait 5 minutes.
- 14. Apply positive pressure for approx. 15 seconds. *(10-15 PSI- Selector to the left).*
- 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
SPE Dry ID: 66819
- 16. Reconstitute in 100µL 100% MeOH and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- 1. Create batch and process data.
- 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 values ≥ 0.98 for each analyte
- 3. RT +/- 3% or 0.100 min, whichever is greater, +/- 20% Accuracy for greater than (+/- 30% for 10ng/ml or less).
Ion ratios must be within +/- 20% of the averaged calibrators
- 4. Case sample response for THC and OH-THC 3ng/mL (quantitative blood), Carboxy-THC: 10ng/mL (qualitative only) will be reported. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
- 5. Did all QCs pass for each analyte? (if not is it describe in comments section)
- 6. Enter QCs into control charting.
- 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: *Extracted on 12/30/19 ratios for THC-COOH were out of range. Re-extracted and ran 1/7/20 THC curve range 3-100, THC-OH evaluated qualitatively only, due to baseline being integrated at lower level requiring a manual integration.*



Toxicology AM method 27 external urine preparation information

Stock solution 8 ul (100 ug/ml) C-THC in 9.992 mls urine
Ppd 11/8/19 Exp: 3/1/20 lot 3120 by AMN

Drug	lot	expiration	lot
C-THC	FE03121501	3/1/2020	3120

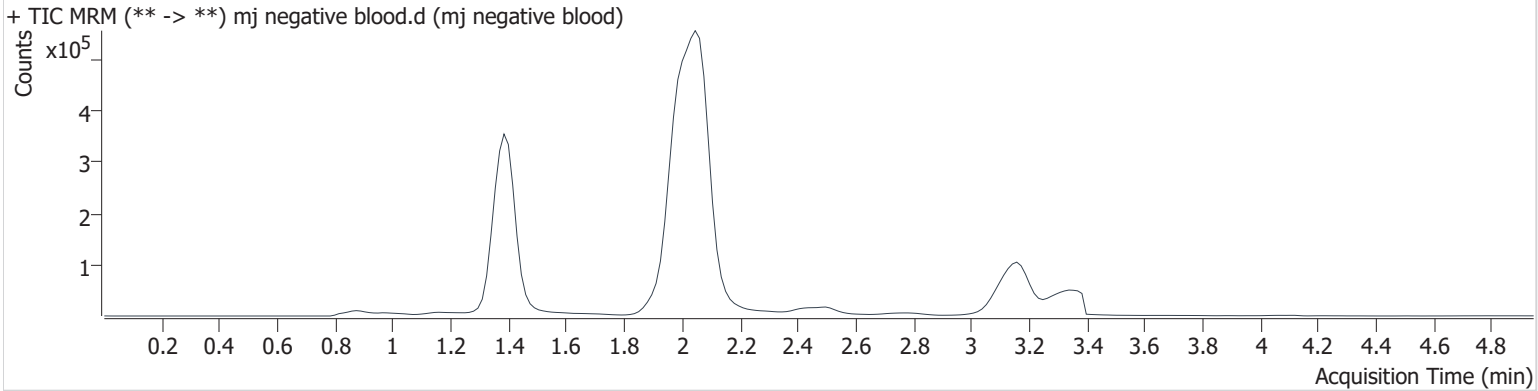
concentration ~ 80 ng/ml Carboxy THC

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj negative blood.d
Type	Sample	Sample	mj negative blood
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A4	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 11:54:49 AM		
Sample Info.			

Sample Chromatogram



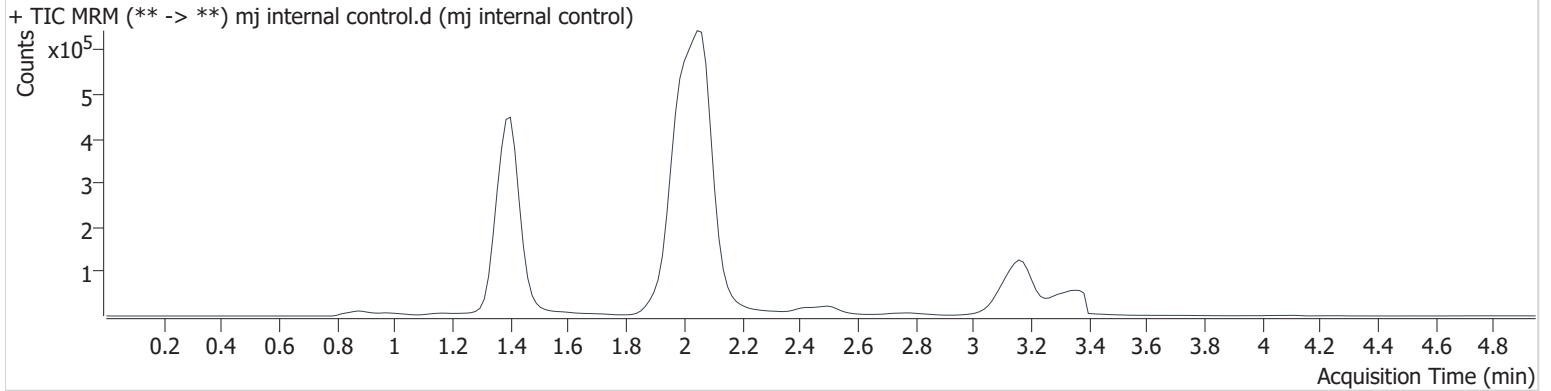
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj internal control.d
Type	QC	Sample	mj internal control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H3	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 1:47:38 PM		

Sample Info.

Sample Chromatogram



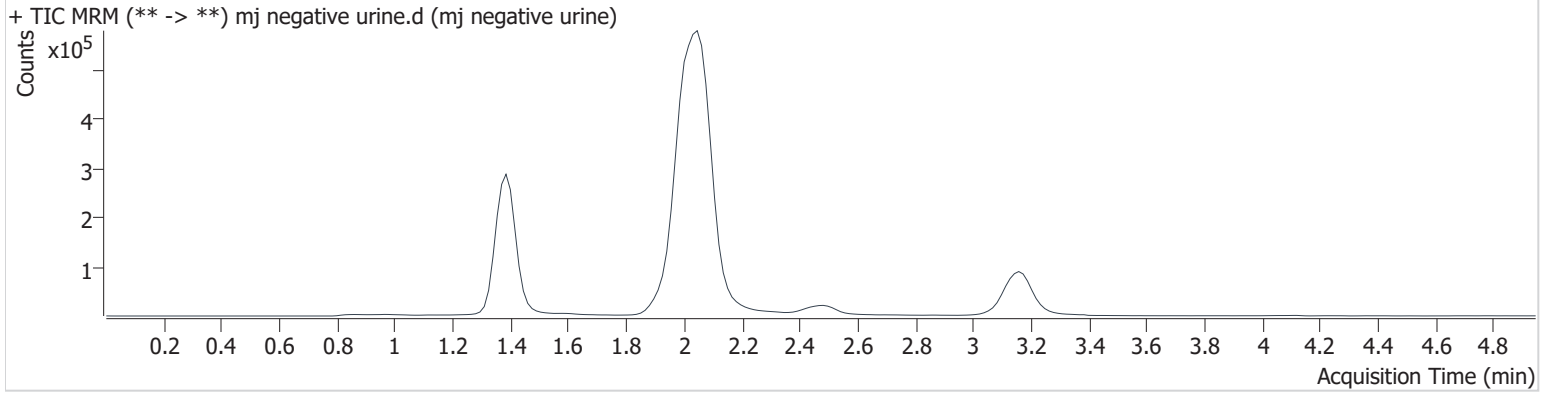
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	102380	157782.7	12.2	25511.0	1461225	4.232 ng/ml
THC-COOH	1.430	107388	252.3	46.0	319.6	613522	14.412 ng/ml
THC	3.183	25488	∞	26.1	253.7	777714	4.415 ng/ml

AM #27 Cannabinoids

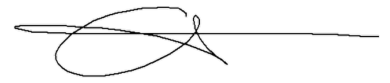
Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj negative urine.d
Type	Sample	Sample	mj negative urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H4	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 3:35:14 PM		
Sample Info.			

Sample Chromatogram



AM #27 Cannabinoids

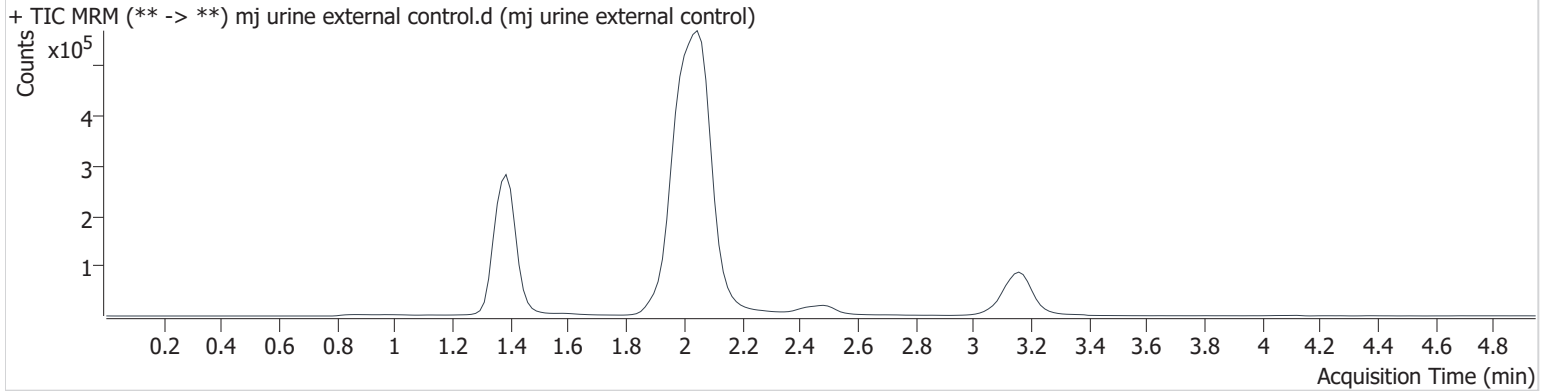


Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj urine external control.d
Type	Sample	Sample	mj urine external control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A5	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 3:42:58 PM		

Sample Info.

Sample Chromatogram

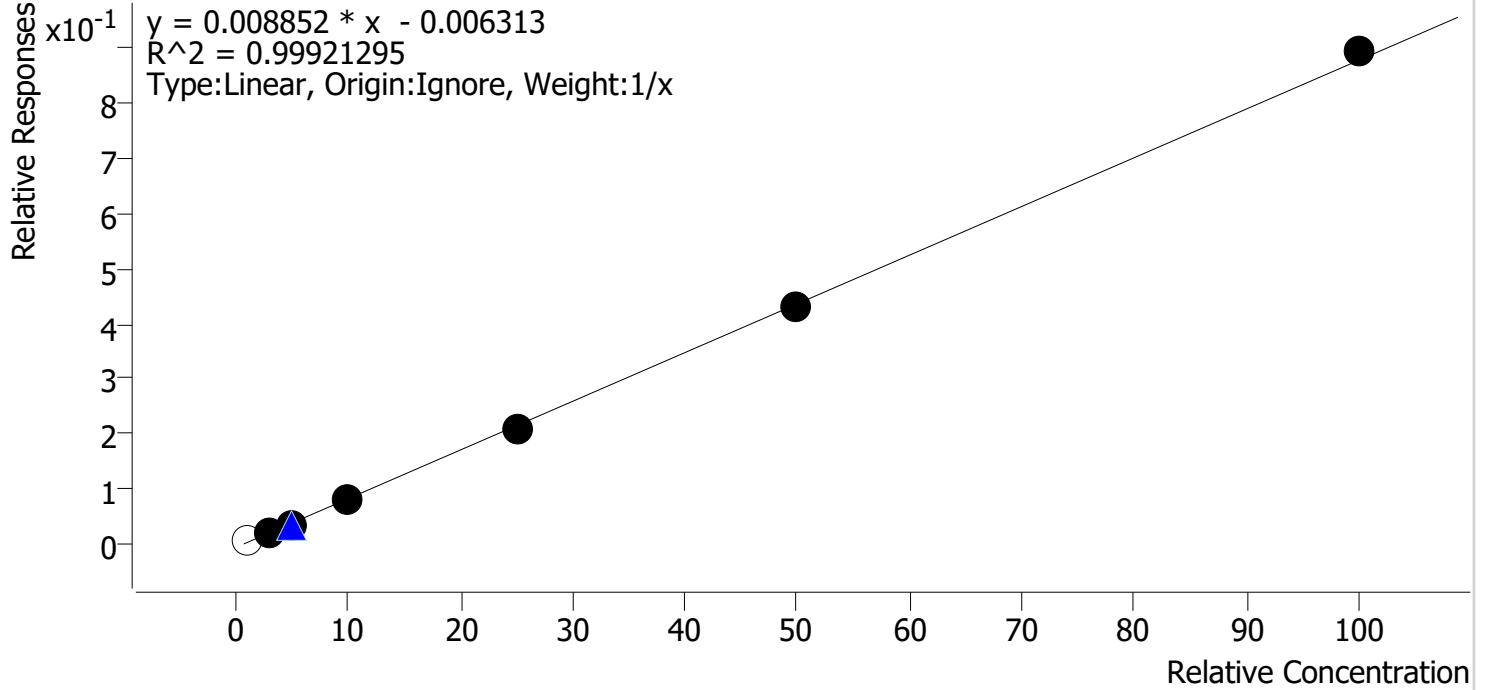


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.415	6285	21.9	51.0	94.0	16091	31.071 ng/ml

Compound Calibration Report

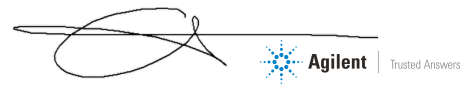
Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Last Cal. Update 1/8/2020 8:44 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

THC - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs



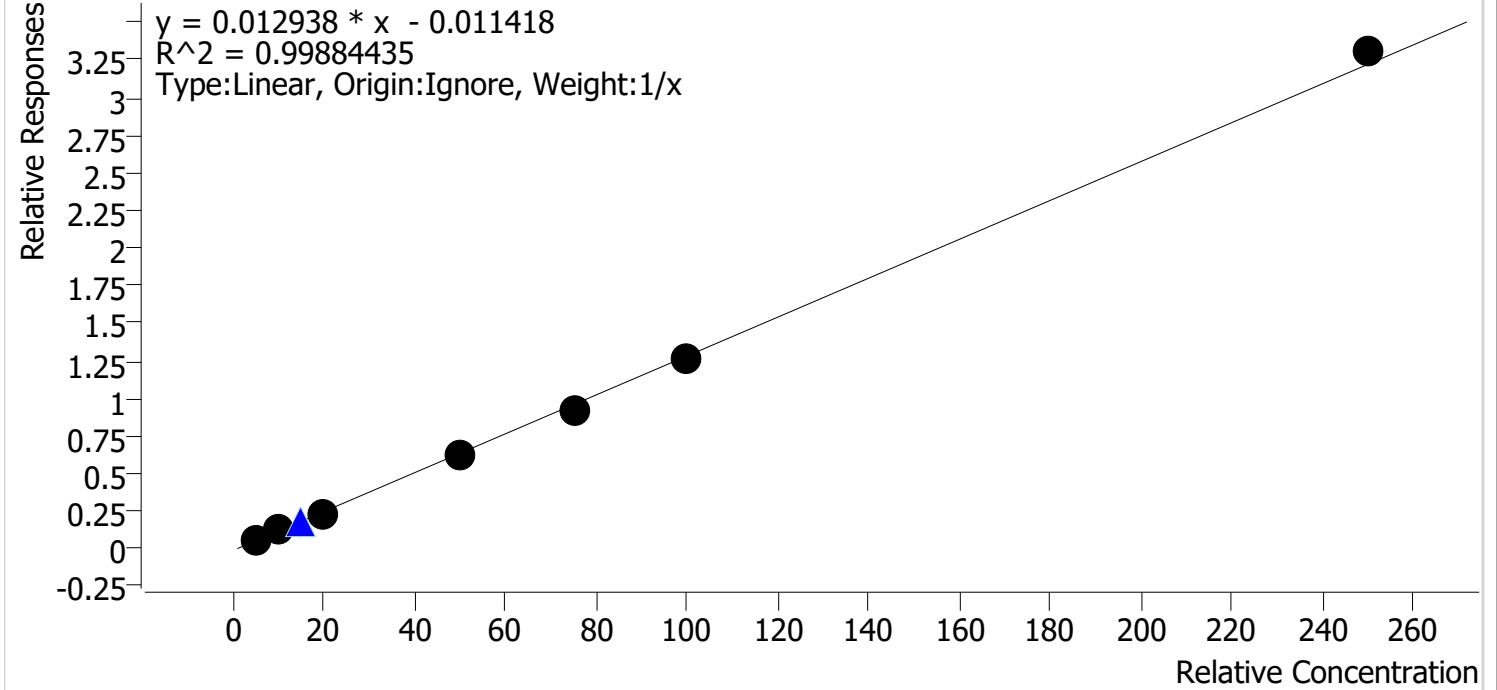
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	x	1.0	1.6	158.4
mj cal2	2	✓	3.0	3.3	108.6
mj cal 3	3	✓	5.0	4.8	96.8
mj cal 4	4	✓	10.0	9.8	98.3
mj cal 5	5	✓	25.0	24.0	95.8
mj cal 6	6	✓	50.0	49.4	98.7
mj cal 7	7	✓	100.0	101.7	101.7

Compound Calibration Report



Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Last Cal. Update 1/8/2020 8:44 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-d9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	5.0	5.5	109.5
mj cal2	2	✓	10.0	10.1	101.3
mj cal 3	3	✓	20.0	18.9	94.5
mj cal 4	4	✓	50.0	49.0	98.0
mj cal 5	5	✓	75.0	72.2	96.3
mj cal 6	6	✓	100.0	97.8	97.8
mj cal 7	7	✓	250.0	256.5	102.6

AM #27 Cannabinoids

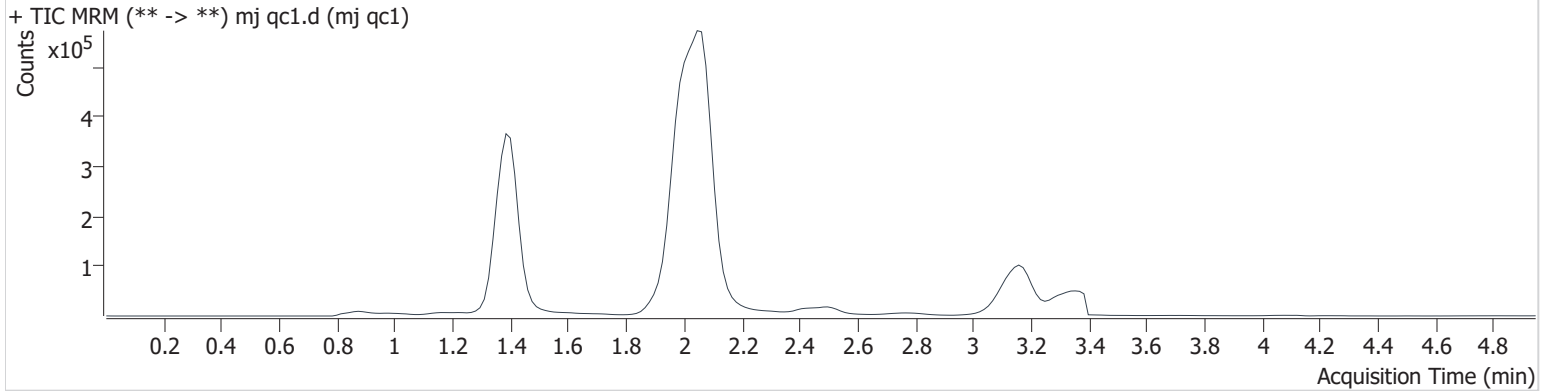


Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj qc1.d
Type	Cal	Sample	mj qc1
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-G3	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 10:53:09 AM		


Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.405	22899	19.3	11.1	14.2	1303464	1.119 ng/ml	Low
THC-COOH	1.415	32804	60.4	46.2	154.6	551897	5.477 ng/ml	Low
THC	3.183	5012	∞	24.9	38.4	649962	1.584 ng/ml	Low

AM #27 Cannabinoids

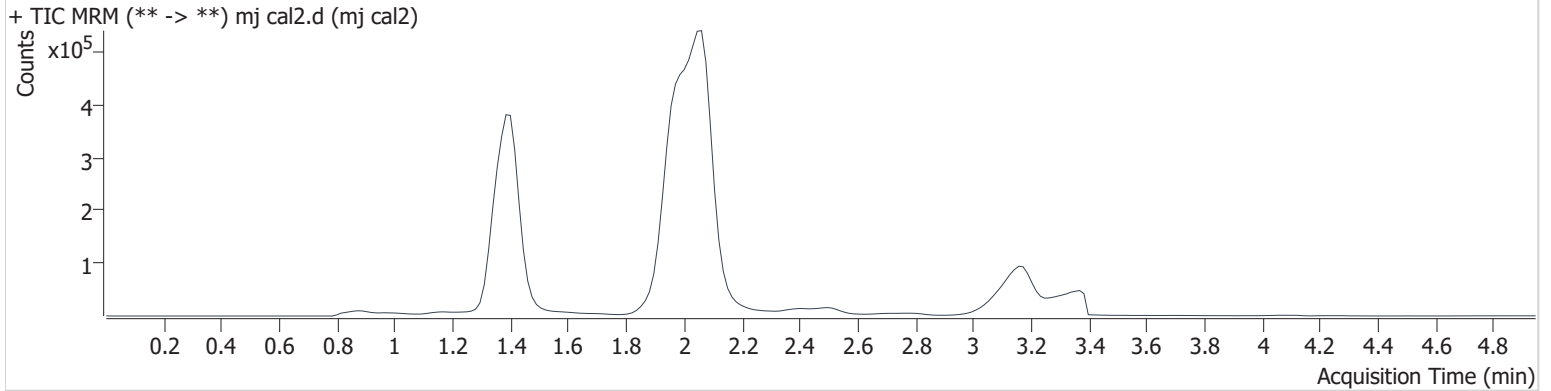


Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj cal2.d
Type	Cal	Sample	mj cal2
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-F3	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 11:00:53 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.405	66514	∞	10.4	18.6	1407459	2.880 ng/ml	Low
THC-COOH	1.415	70156	119.1	43.7	67.0	586602	10.127 ng/ml	
THC	3.183	14034	∞	29.1	67970 31919 175.4	623207	3.257 ng/ml	

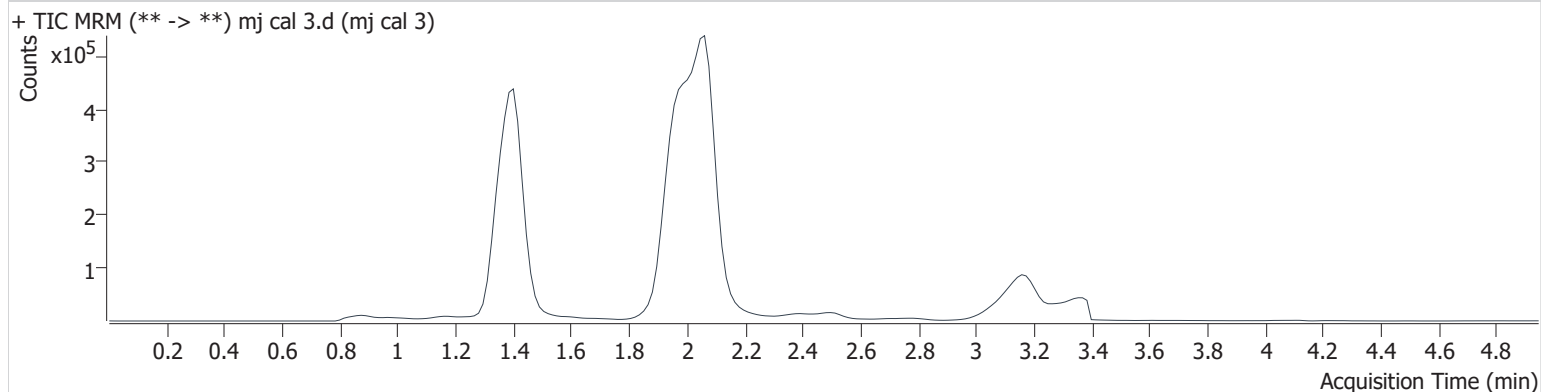
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj cal 3.d
Type	Cal	Sample	mj cal 3
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-E3	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 11:08:35 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.405	107967	∞	12.9	32168.9	1497121	4.354 ng/ml
THC-COOH	1.415	143586	212202.2	47.8	1773.8	615933	18.901 ng/ml
THC	3.183	21343	∞	28.2	∞	583923	4.842 ng/ml

AM #27 Cannabinoids

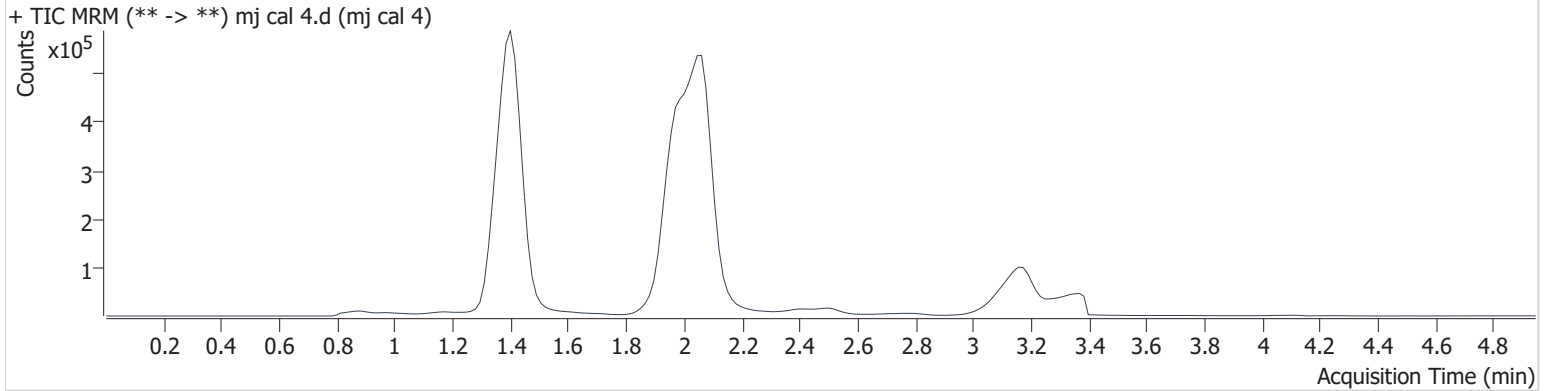


Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj cal 4.d
Type	Cal	Sample	mj cal 4
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-D3	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 11:16:17 AM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	263256	315986.7	10.4	43716.7	1485759	10.584 ng/ml
THC-COOH	1.415	368195	1322.2	48.2	3317.6	591334	49.010 ng/ml
THC	3.183	51371	∞	25.0	∞	636716	9.827 ng/ml

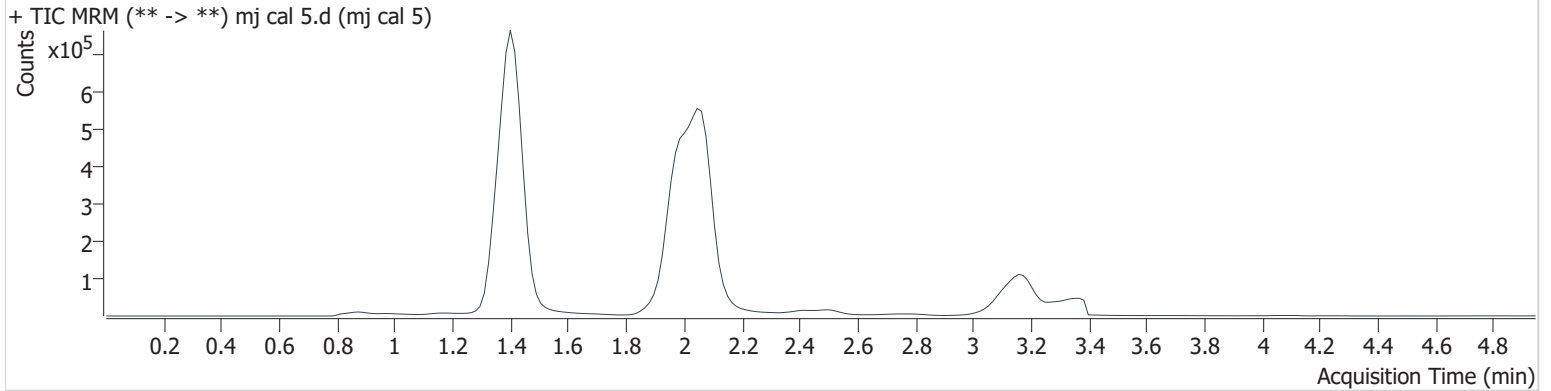
AM #27 Cannabinoids



Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj cal 5.d
Type	Cal	Sample	mj cal 5
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-C3	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 11:23:59 AM		
Sample Info.			

Sample Chromatogram



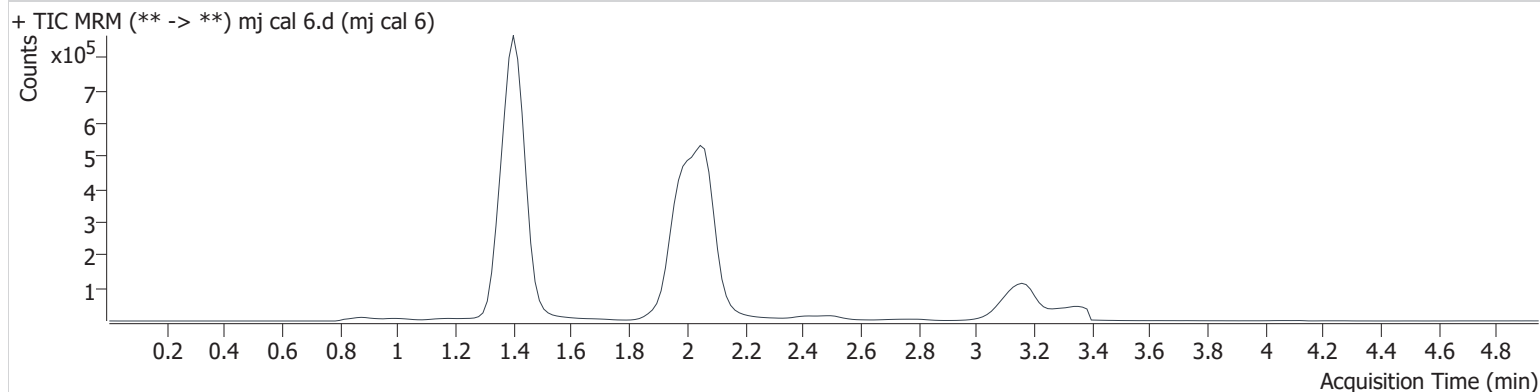
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	600635	1379485.4	11.2	126.5	1447603	24.682 ng/ml
THC-COOH	1.415	546092	1334.9	49.0	2164.1	591534	72.239 ng/ml
THC	3.183	128762	∞	25.2	∞	625687	23.960 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj cal 6.d
Type	Cal	Sample	mj cal 6
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-B3	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 11:31:41 AM		
Sample Info.			

Sample Chromatogram



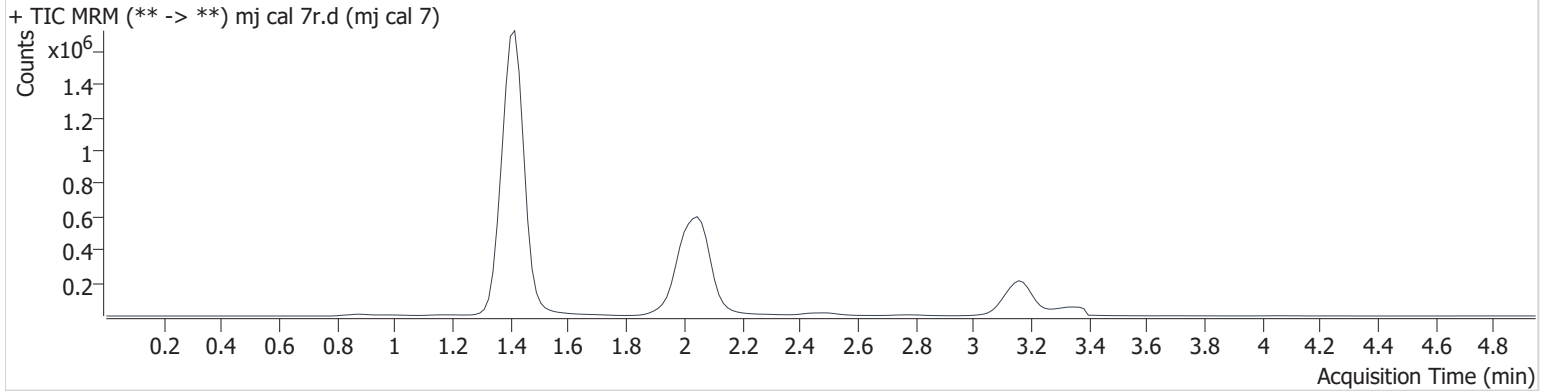
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	1040382	1334197.0	11.4	746.2	1233174	50.108 ng/ml
THC-COOH	1.415	635777	2295.9	48.4	3212.9	507269	97.758 ng/ml
THC	3.183	233276	∞	25.0	∞	541614	49.368 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\AM 27\010719\QuantResults\cann quant.batch.bin
Calibration Last Update 1/8/2020 8:44:20 AM

Instrument	69679	Data File	mj cal 7r.d
Type	Cal	Sample	mj cal 7
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A3	Comment	
Injection Volume	10		
Acq. Date-Time	1/7/2020 12:25:35 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	2055790	7008531.0	12.1	76557 0.0	1216723	100.274 ng/ml
THC-COOH	1.415	1580009	22676.6	49.8	11647. 7	477787	256.489 ng/ml
THC	3.183	575891	∞	26.1	∞	643901	101.746 ng/ml